1-17-91

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008231

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Kathon 886 F Microbicide (EPA Reg. No. 707-130)
Submission of Microbial Mutagen Assay Under FIFRA
Section 6(a)(2)

TO: John Lee
Product Manager (31)
Registration Division (H7508C)

FROM: Linda L. Taylor, Ph.D.
Toxicology Branch II, Section II
Health Effects Division (H7509C)

Thru: K. Clark Swentzel.
Toxicology Branch II, Head Section II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D.
Chief, Health Effects Division (H7509C)

Registrant: Rohm & Haas Company
Chemical: 5-chloro-2-methyl-4-isothiazolin-3-one, 2 methyl-4-isothiazolin-3-one
Synonyms: Kathon 886 F
Project: 1-0227
Caswell No.: 195C
Record No.: not provided; Case # 0-22081

Action Requested: Please review microbial mutagen assay.

Comment: The Registrant submitted this mutagenicity assay under FIFRA Section 6(a)(2) because the results indicate that Kathon 886 F microbicide is mutagenic in Salmonella tester strains TA98 and TA1537 in the presence of metabolic activation. These results differ from previous assay results, which indicated that Kathon 886 F was mutagenic only in tester strain TA100 without metabolic activation (MRID # 105044). The Registrant contends that this change in activity is attributed to a shortening of the S-9 metabolic activating system preincubation time and not to a change in product composition.

The assay has been evaluated and is summarized below. The DER is attached.

Under the conditions of the assay, Kathon 886 F is mutagenic in the Salmonella tester strains TA98 and TA1537 in the presence of metabolic activation and toxic at all concentrations of the test material utilized without metabolic activation.

The Registrant states that this new finding does not alter significantly the risks associated with the currently registered uses of Kathon 886 F microbicide. Additionally, the negative findings in the Drosophila sex-linked recessive assay, in a cell transformation assay, and in multiple cytogenetic tests were cited as more appropriate assays for evaluating the mutagenic potential of this type of compound (antimicrobial).

The study submitted here is in agreement with other gene mutation assays with this test material; i.e., Kathon 886 is mutagenic in the gene mutation assay. This does not alter Kathon 886 status. However, as noted in the TB II memo of 10/1/90, the test material used in the various studies submitted to fulfill the data requirements for Kathon 886 appears to vary in composition. In many cases the active ingredients are outside their certified limits and the total percent active ingredient is higher than is stated to be stable. In the current assay, there was no information provided on the percent of each component, and the percent total active ingredient (14%) is greater than the highest active ingredient level (13.1%) said to be stable. As requested previously (TB II memo dated 10/1/90), the Registrant should address these aspects of their test material.

The assay is acceptable when considered with the other similar studies on this test material. As determined previously (TB II memo dated 4/9/90), the data requirements for mutagenicity for Kathon 886 are (1) structural chromosomal aberration and (2) other genotoxic effects. The Registrant has indicated that studies will be performed to satisfy these two categories.

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Reviewed by: Linda L. Taylor, Ph.D. 1/4/91
Tox. Branch II, Section II, (H7509C)
Secondary reviewer: K. Clark Swentzel 1/7/91
Tox. Branch II, Section II, (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity - Salmonella; Ames Assay TOX. CHEM. NO.: 195C

MRID NO.: 415752-01

TEST MATERIAL: 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one

SYNONYMS: Kathon 886

STUDY NUMBER: Report #: 90R-0142

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Rohm & Haas Company, Toxicology Department

TITLE OF REPORT: Kathon 886: Salmonella Typhimurium Gene Mutation Assay (Screening test in TA98 and TA1537)

AUTHORS: JL Sames and JP Frank

REPORT ISSUED: 7/24/90

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the assay, Kathon 886 is mutagenic in the Salmonella tester strains TA98 and TA1537, with metabolic activation. The test material was toxic at all concentrations without metabolic activation. After pre-incubation of the test material with an S-9 activation mixture, the mutagenic activity was either slight (at 60 ug/plate, HDT; significant only in TA1537) or not detected.

Classification: Unacceptable; this was a screening test (the test material is not adequately identified and only two tester strains were studied). There are other Ames assays available on this test material and the results agree; i.e., Kathon 886 is mutagenic in the gene mutation assay. For the record, the test material needs to be adequately identified. Available data satisfy the guideline requirements [84-2 (a)] for gene mutation, Category I.

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A. MATERIALS

1. Test Material: Name: Kathon 886; 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one; Description: none provided; Batch #: Lot #: 2585; Solvent used: distilled water; Purity: 14%; unless otherwise indicated, all concentrations were adjusted for a.i.. NOTE: the % of each component was not indicated.

2. Control Materials:
Negative:
Solvent/final concentration: DMSO, except for 9-aminoacridine (95% ethanol)/ ?????
Positive: Non-activation:
2-Nitrofluorene 3 ug/plate TA98
9-Aminoacridine 100 ug/plate TA1537

Activation:
2-Aminoanthracene (2-anthramine) 2 ug/plate (both strains)

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The S-9 consisted of the following:

Nicotinamide-adenine dinucleotide phosphate (NADP)	4×10^{-3} mole
Glucose-6-phosphate	5×10^{-3} mole
Magnesium Chloride ($mgCl_2$)	8×10^{-3} mole
Potassium chloride (KCl)	33×10^{-3} mole
Sodium phosphate buffer, pH 7.4	100×10^{-3} mole
Liver homogenate (S-9) from Aroclor 1254-induced rats	10 %

The non-activated portion of the assay consisted of the above mix with equal amounts of saline substituted for NADP and S-9.

4. Test organisms: S. typhimurium strains
___ TA97 X TA98 ___ TA100 ___ TA102 ___ TA104
___ TA1535 X TA1537 ___ TA1538; list any others.
The above strains were obtained from B. Ames of Berkeley, CA and were characterized (for nutritional requirements, crystal violet sensitivity, and ampicillin resistence) no more than 6 months prior to initiation of the study.

5. Test compound concentrations used:
Non-activated conditions: 1.6, 3, 5, 9, 16, and 30 ug/plate, initially; up to 60 ug/plate in an independent assay. All concentrations were adjusted for active ingredient.
Activated conditions: same as for non-activated conditions.

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B. TEST PERFORMANCE

1. Type of Salmonella assay:
- X standard plate test
- X pre-incubation (120 minutes)
- ___ "Prival" modification (i.e., azo reduction method)
- ___ spot test
- ___ other (describe)

The tester strains were sub-cultured in Oxoid Nutrient Broth # 2 for approximately 10 hours at 37°C, and the fresh inoculum was used when bacterial growth was approximately 10^8 to 10^9 cells per mL. Control plates were run to check for sterility, determine the background reversion rate, and measure the response of each tester strain to a positive control compound.

The following were added (in order) to sterile test tubes for (1) the activated portion of the assay: 2 mL top agar, 0.1 mL bacteria inoculum, 0.1 mL of the appropriate concentration of test material, and 0.5 mL phosphate buffer mix (with S-9 and NADP; (2) the non-activated portion of the assay: the above procedure was followed except that the 0.5 mL phosphate buffer mix was added to the tubes directly after the top agar.

Each test material concentration was tested in triplicate, in minimal plates (minimal - glucose agar medium). The controls were tested in six replicates in minimal plates. The contents of the tubes were mixed and poured onto petri dishes containing approximately 19 mL of the appropriate agar. The plates were allowed to set for several minutes, then placed in covered plastic boxes and incubated at 37°C for approximately 72 hours prior to colony counting.

For the pre-incubation portion of the study, the test material was mixed with S-9 and held for 2 hours at room temperature, prior to adding top agar and bacteria.

2. Preliminary cytotoxicity assay: There was no preliminary cytotoxicity assay as such; i.e., none was reported. However, the results of the assay in the absence of metabolic activation showed that the test material was toxic at all concentrations used (see below).

With regard to the dose levels used, the author indicated that since this assay was designed to evaluate mutagenic activity at specific concentrations, it was not considered necessary to carry out experiments to the usual limits (5000 ug/plate, limit of solubility, toxicity).

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3. Mutagenicity assay: A mutagenic response was detected in both tester strains in the presence of metabolic activation. In the absence of metabolic activation, the test material was toxic at all concentrations tested (see Table I, copy attached). The results of the activated assay were confirmed in an independent assay in which concentrations up to 60 ug/plate were used (see Table II, copy attached). NOTE: In the first assay, 9 ug/plate was positive, but in the confirmation assay, 10 ug/plate was negative.

   An additional assay, designed to monitor the effects of S-9 pre-incubation, produced contrasting results. In both strains, all concentrations (not toxic in the standard protocol) were negative (see Table III, copy attached). At 60 ug/plate (toxic in the standard protocol), the test material was non-toxic in both strains. A slightly greater than 2-fold increase (over background)* in revertants was detected in TA1537, using the pre-incubation technique. The pre-incubation assay was performed only once.

   *response considered positive if test material elicited a two-fold increase in the # of revertants/plate over that of the solvent control

4. Reviewer's discussion/conclusions: The test material had been found (Scribner, 1983; Report # 81R-97) to be mutagenic in the Ames assay but only in tester strain TA100 and only under non-activated conditions. More recently, Kathon DP-3 (of which Kathon 886 is a major component) was found to be positive in all four tester strains (TA98, TA100, TA1535, and TA1537) in the presence of metabolic activation (Report # 89R-0267). The objective of the current study was to assess the potential of Kathon 886 to induce gene mutations, in strains other than TA100, in the presence of metabolic activation and to assess the mutagenic potential following S-9 pre-incubation. Under the conditions of this assay, Kathon 886 is mutagenic in the Salmonella gene mutation assay. When the test material was preincubated with an S-9 activation mixture, the activity was either slight or not detected.

   It is to be noted that no description of the test material was provided, and the percent of each component of the test material was not indicated. As in previous TB II memos on this test material (dated 4/9/90 and 10/1/90), TB II points out that the test material has not been adequately identified. Additionally, the 14% a.i. used in this assay is greater than that previously identified by the Registrant as stable; i.e. 13.1%.

   Although this assay (screening test) does not meet the acceptance criteria for a gene mutation assay (the test material is not adequately identified and only two tester strains were studied), there are other Ames assays available on this test material and the results agree; i.e., Kathon 886 is mutagenic in the gene mutation assay. For the record, the test material needs to be adequately identified.

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